Association of Intrinsic Variants of the BTBD9 Gene
With Tourette Syndrome

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Objective: To test the association between Tourette syndrome (TS) and genetic variants in genomic loci MEIS1, MAP2K5/LBXCOR1, and BTBD9, for which genome-wide association studies in restless legs syndrome and periodic limb movements during sleep revealed common risk variants.

Results: The study provided 3 single-nucleotide polymorphisms within BTBD9 associated with TS ($\chi^2=8.02$, $P=.005$ for rs9357271), with the risk alleles for restless legs syndrome and periodic limb movements during sleep overrepresented in the TS cohort. We stratified our group of patients with TS according to presence or absence of obsessive-compulsive disorder and/or attention-deficit disorder and found that variants in BTBD9 were strongly associated with TS without obsessive-compulsive disorder ($\chi^2=12.95$, $P<.001$ for rs9357271). Furthermore, allele frequency of rs9357271 inversely correlated with severity of obsessive-compulsive disorder as measured by the Yale-Brown Obsessive Compulsive Scale score.

Conclusion: Variants in BTBD9 that predispose to restless legs syndrome and periodic limb movements during sleep are also associated with TS, particularly TS without obsessive-compulsive disorder.

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Methods

Restless Legs Syndrome (RLS) is a neurological condition characterized by a recurrent urge to move the legs, with or without an uncomfortable sensation.\(^1\)\(^2\) Periodic limb movements during sleep (PLMS), which consist of repetitive and stereotyped movements of the legs, are frequently found in patients with RLS.\(^3\) Tourette syndrome (TS) is a neurodevelopmental disorder characterized by motor and vocal tics,\(^2\) often accompanied by attention-deficit disorder (ADD) with or without hyperactivity and obsessive-compulsive disorder (OCD).\(^5\)\(^7\)

Although RLS and TS are distinct movement disorders, they share some common features, and a high prevalence of RLS has been reported in TS.\(^8\)\(^9\) In addition, RLS and TS have been linked to dysfunction in frontostriatal circuits, and both are responsive to modification of dopamine neurotransmission.\(^10\)\(^11\) Sensations relieved by movement are also found in TS cases; patients frequently describe urges or sensations preceding tics.\(^12\) Although it is still unclear whether TS has a sensory component, this question is receiving great attention because sensory phenomena may help to identify more homogeneous subgroups of TS and OCD cases.\(^13\)

Recently, a genome-wide association study identified the following 3 loci associated with RLS: MEIS1 (OMIM 601739), BTBD9 (OMIM 611237), and MAP2K5/LBXCOR1 (OMIM 602520/611273).\(^14\) Simultaneously, another genome-wide association study uncovered an association between variants in BTBD9 and PLMS.\(^15\)

To assess the role of these genes in TS, we analyzed 14 single-nucleotide polymorphisms (SNPs) spanning the 3 loci and reported to be associated with RLS.\(^14\) We performed a case-control and a family-based association study and found that the predisposing alleles for RLS in the BTBD9 gene are associated with TS, particularly TS without psychiatric comorbidities.

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Group Information: The Montreal Tourette Study Group members are listed at the end of this article.

SAMPLE COLLECTION

Two hundred ninety-eight French Canadian TS trios (father, mother, and proband) were re-
cruited through the Montreal General Hospital, Montreal, Que-
bec, Canada, and Sainte Justine Hospital for the purpose of family-
based association studies. Exclusion criteria were (1) the inability
to provide a consent form, (2) a history of another neurological
disorder, and (3) the presence of tics induced by drugs or other
factors. Ethics committee approval and informed consent were
obtained. Experienced clinicians performed diagnostic evalua-
tions of TS, chronic tics, OCD, and ADD during direct family in-
terviews. Tourette syndrome, chronic tics, and ADD were diag-
nosed using criteria of the
Diagnostic and Statistical Manual of
Mental Disorders
(Fourth Edition).

Obsessive-compulsive disorder was evaluated using the Yale-Brown Obsessive
Compulsive Scale (YBOCS) and classified as absent or present (0/11021
points). Our cohort of TS cases consists of 298 unrelated
probands originating from the collection of trios and 24 unre-
lated additional cases, for a total of 322 TS cases. Two hundred
ninety unrelated healthy, ethnically matched individuals were used
as control subjects for this group of patients. Genomic DNA was
extracted from whole blood following standard methods.

SNP GENOTYPING

We selected 14 SNPs found to be associated with RLS or PLMS
in the 2 genome-wide association studies (Table 1).

Table 1. Selected SNPs and Association Results in RLS From Previous Studies

<table>
<thead>
<tr>
<th>SNP Information</th>
<th>Association Results in RLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome</td>
<td>Gene</td>
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<tr>
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<td>MEIS1</td>
</tr>
<tr>
<td>2p</td>
<td>MEIS1</td>
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<tr>
<td>6p</td>
<td>BTBD9</td>
</tr>
<tr>
<td>6p</td>
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</tbody>
</table>

Abbreviations: dbSNP, single-nucleotide polymorphism (SNP) database; ID, identification; MAF, minor allele frequency; RLS, restless legs syndrome.

* Indicates overrepresented allele in patients with RLS.

Table 2. Case-Control Association Results

<table>
<thead>
<tr>
<th>SNP No.</th>
<th>dbSNP ID</th>
<th>Genotyping Success Rate, %</th>
<th>HWE P Value</th>
<th>Alleles, Major/Minor</th>
<th>Risk Allele</th>
<th>MAF, Case/Control</th>
<th>( \chi^2 ) Value</th>
<th>P Value</th>
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<tbody>
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<td>1</td>
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<td>0.26/0.23</td>
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<td>0.26/0.25</td>
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<td>A/G</td>
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<td>G</td>
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<td>0.21</td>
<td>.64</td>
</tr>
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</table>

Abbreviations: dbSNP, single-nucleotide polymorphism (SNP) database; HWE, Hardy-Weinberg equilibrium test; ID, identification; MAF, minor allele frequency.

* Indicates uncorrected. In the Bonferroni correction for multiple testing, the significance level was set to .0127, as explained in the “Statistical Analyses” subsection of the “Methods” section. Boldface \( P \) values are less than the significance threshold.

STATISTICAL ANALYSES

Case-control association analysis, a transmission disequilibrium test, a Hardy-Weinberg equilibrium test, and pairwise linkage disequilibrium calculations were performed using Haploview, version 4.1.10 Haplotype blocks were defined using the method of Gabriel et al10 and implemented in Haploview. Single-factor analysis of variance was performed on YBOCS total scores.
based on genotype subgroups obtained for rs9357271 (T/T, T/C, and C/C). Because of the strong linkage disequilibrium between the SNPs, a simple Bonferroni correction for multiple testing would have resulted in an overly conservative test. The association results from the 14 SNPs were therefore adjusted for multiple testing by using the method of Li and Ji\textsuperscript{20} for the effective number of independent tests, and the significance level was set to .0127 = 1−(1− 0.05)\textsuperscript{0.25}. We further corrected for multiple testing using the Bonferroni method for the case-control analysis of rs9357271 in different TS subgroups. The significance level was set to .00254 = .0127/5, with n = 5 tests (TS + OCD, TS − OCD, TS + ADD, TS − ADD, and TS − OCD − ADD).

The genotyping success rate of the 14 SNPs ranged from 98.5% to 99.8%. As another quality control, Hardy-Weinberg equilibrium tests were performed, and the results revealed no significant deviation from this equilibrium (Table 2). Pairwise linkage disequilibrium measured by global D’ indicated that the markers spanning MEIS1, BTBD9, and MAP2K5/LBXCOR1 are in strong linkage disequilibrium at each locus (Figure, A). Minor allele frequencies (MAFs) from our control group

Figure. Findings in patients with Tourette syndrome (TS) with and without psychiatric comorbidities. A, Pairwise linkage disequilibrium diagrams for the 3 loci (MEIS1, BTBD9, and MAP2K5/LBXCOR1) with risk variance for restless legs syndrome. Pairwise linkage disequilibrium was measured as D’ and calculated from our case-control cohort, in Haploview, using the methods of Gabriel et al.\textsuperscript{19} The white-to-red gradient reflects lower to higher linkage disequilibrium values, respectively; kb indicates kilobase. Single-nucleotide polymorphisms (SNPs) in bold listed across the top indicate haplotype blocks. These SNP alleles show strong linkage disequilibrium among themselves, and there is little evidence of recombination. B, Graphical representation of minor allele frequencies (MAFs) of rs9357271 in control subjects and in subgroups of patients, derived from the MAF values in Table 2. From left to right on the x-axis, subgroups of patients are aligned from highest to lowest MAF. C, Graphical representation of MAFs of rs9357271 in subgroups of patients classified according to Yale-Brown Obsessive Compulsive Scale (YBOCS) scores as having moderate obsessive-compulsive disorder (OCD) (YBOCS score of 16-23; MAF, 0.18; 64 individuals) and severe OCD (YBOCS score of ≥24; MAF, 0.24; 39 individuals). The TS − OCD subgroup is shown on the right (YBOCS score of <16; MAF, 0.14; 188 individuals). ADD indicates attention-deficit disorder with or without hyperactivity.
Minor allele frequencies of the SNPs within MEIS1 and MAP2K5/LBXCOR1 did not significantly differ between TS cases and controls (SNPs 1 and 2 and SNPs 8-14) (Table 2). However, MAFs of rs4714156, rs9296249, and rs9357271, all located within intron 7 of BTBD9, were significantly lower in TS cases than in controls (0.17 vs 0.23 \( P = 0.007 \), 0.16 vs 0.22 \( P = 0.01 \), and 0.17 vs 0.23 \( P = 0.005 \), respectively). With rs9357271 being the most strongly associated SNP (Table 2).

We divided our cohort of patients according to the presence or absence of ADD and OCD to compare the MAFs of the most significantly associated SNP (rs9357271) in cases with (TS + OCD and TS + ADD) and without (TS−OCD, TS−ADD, and TS−OCD−ADD) the 2 most prevalent psychiatric comorbidities observed in TS. The graphical representation of MAF (Figure, B) illustrates the important variation in allele frequency of rs9357271 between subgroups of patients with TS, which are aligned from highest to lowest MAF. The number of cases, MAF, and association results of each of the 5 TS subgroups are presented in Table 3. Minor allele frequencies determined for the TS + ADD subgroups were comparable to those found in the TS cases and the RLS cohort (0.17, 0.17, and 0.16, respectively) (Tables 1, 2, and 3), whereas the TS−OCD, TS−ADD, and TS−OCD−ADD subgroups yielded lower MAFs (0.14, 0.14, and 0.10, respectively) (Table 3). The TS + OCD subgroup presented the highest MAF (0.20) (Table 3). Concerning the association results, only the TS−OCD and TS−OCD−ADD subgroups reached the significance level of 0.00254 after Bonferroni correction for multiple testing.

The total YBOCS score is a measure of severity for patients with OCD. Patients with scores ranging from 16 to 23 are considered to have moderate OCD, and patients with scores of 24 or higher are severely affected. As shown in the Figure, B, and Table 3, TS cases with a YBOCS score of 16 or higher (TS + OCD) have a higher MAF than do TS cases with a YBOCS score of less than 16 (TS−OCD) (0.20 vs 0.14). The TS + OCD subgroup (103 patients) was then subdivided according to severity into moderate OCD (YBOCS score 16-23; 64 individuals) and severe OCD (YBOCS score ≥24; 39 individuals) (Figure, C). There was a significant difference in total YBOCS scores among the 3 genotype subgroups of rs9357271 (T/T, T/C, and C/C) (1-way analysis of variance, \( F_2=3.38 \) \( P =.04 \)).

### FAMILY-BASED ASSOCIATION RESULTS

As demonstrated in the case-control analysis, SNPs in MEIS1 and MAP2K5/LBXCOR1 were not found to be associated with TS. The major alleles of the 3 SNPs within BTBD9—rs4714156 (C), rs9296249 (T), and rs9357271 (T), which are the predisposing alleles in RLS—were found to be transmitted from parents to the progeny with TS, although the P values were not significant after correction for multiple testing (Table 4). The
observed overtransmission of the risk allele of rs9357271 in the family-based study mainly originated from the trios with probands who had TS−OCD (transmitted, 61; nontransmitted, 38) compared with the rest of the cohort (transmitted, 45; nontransmitted, 38) (data not shown).

**COMMENT**

By performing a case-control association study in the French Canadian population, we found a significant association between genetic polymorphisms within BTBD9 and TS. The same overrepresented alleles in our TS cohort also predispose to RLS and PLMS. Interestingly, BTBD9 was the only gene significantly associated with RLS and PLMS in the 2 independent genome-wide association studies. This study provides evidence of a molecular genetic link between RLS and TS. However, this is not the first report of such common genetic variants accounting for the genetic predisposition of different clinical phenotypes, which may implicate common shared molecular pathways or impaired structured functions underlying different phenotypes. For instance, common variants in intron 5 of the CDKAL1 gene have been associated with psoriasis, Crohn disease, and type 2 diabetes mellitus.

The most significant SNP in BTBD9 (rs9357271 [P = .005]) was further analyzed to verify the presence of more specific genotype-phenotype correlations in TS with and without OCD and ADD, which are the most prevalent TS comorbidities observed in clinical practice. Strikingly, MAFs of rs9357271 were found to be even lower in TS cases without OCD, ADD, or either of them than in the TS and RLS cohorts. However, only the TS−OCD (MAF, 0.14 [P < .001]) and the TS−OCD−ADD subgroups (MAF, 0.10 [P = .002]) reached the significance level for association after Bonferroni correction for multiple testing. We also found that the allele frequency of rs9357271 inversely correlated with OCD severity, as defined by the total YBOCS score, which further supports the association between variants in BTBD9 and individuals with TS−OCD. Our results indicate that genetic polymorphisms within BTBD9 may play a more significant role in the etiology of TS without psychiatric comorbidities (also termed pure TS) and particularly in TS without OCD. Bidirectional overlap between TS and OCD is well documented. Although TS + OCD is thought to constitute a form of TS rather than a form of OCD, patients with TS + OCD and those with TS−OCD present important phenotypic dissimilarities. Thus, one may argue that genes underlying TS + OCD may at least partially differ from genes predisposing to TS alone. The fact that TS lies at the interface of neurology and psychiatry, together with the assumption that BTBD9 predisposes to 3 distinct movement disorders (TS, RLS, and PLMS), led us to hypothesize that BTBD9 is more specifically involved in the neurological component of TS and in the circuitry leading to abnormal movements than in the etiology of TS-related psychiatric behaviors.

We are aware that our study has several limits, including the relatively small number of cases and controls. However, the SNP MAFs found in our control group are very similar to the ones obtained in the original study. The fact that we work with a population isolate (French Canadian samples) should also decrease the risk of case-control mismatch. Unfortunately, our clinical evaluation of TS does not include PLMS measurement, and it is still unknown whether PLMS is more frequently observed in patients with TS who have no psychiatric comorbidities. The described association between TS and BTBD9 is unlikely to be explained by the RLS comorbidity in these patients with TS because RLS was found in only 10% of these cases. The association between TS and BTBD9 needs to be replicated in independent samples with the same phenotype and subphenotype classifications.

Although we observed an overtransmission of the BTBD9 risk alleles from parents to probands with TS assessed by using a family-based association approach (with P < .05), this distortion in transmission did not reach the significance level for association after correction for multiple testing. We estimate that this lack of significant association is due to the complex inheritance pattern of TS and its comorbidities, which have a greater negative effect on family-based studies than on case-control analyses.

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